Abstract – Phenotypic and genotypic characterization of virulence traits of staphylococci of animal origin

Staphylococci can express a wide array of virulence traits which may strongly influence the infection prognosis, often being a multidrug resistant animal pathogen, responsible for conditions such as abscesses, dermatitis or mastitis. In this study we aimed to perform a phenotypic and genotypic characterization of virulence traits of methicillin-resistant staphylococci (MRS) isolates of animal origin. A total of 253 staphylococci were evaluated for methicillin resistance. This collection comprises isolates from bovine clinical and subclinical mastitis and other clinical isolates from dogs, cats, horses and goats. Identification had been previously performed by biochemical tests and confirmed to genus or species level by PCR and isolates clonality was evaluated by PFGE. Methicillin resistance screening was performed by the oxacillin disc diffusion according to CLSI guidelines and confirmed using a MRSA modified medium and by PCR amplification of the *mec*A gene. The occurrence of the new *mec*A homolog, LGA_{251} was evaluated by PCR in *mec*A negative MRS isolates. Virulence traits of MRS such as coagulase, haemolysins, DNase, gelatinase and lipase were phenotypically evaluated. Biofilm production was also phenotypically evaluated and by PCR of *ica*A, *ica*D and *bap* genes. Quorum-sensing system *agr* was determined by PCR. Antimicrobial susceptibility to 18 compounds was evaluated according to CLSI guidelines. The presence of bacteriophages and the production of bacteriocins were determined by plaque assays.

A total of 26 isolates were identified as MRS: *S. epidermidis* (n=16), *S. aureus* (n=4) and *Staphylococcus* spp. (n=6). From the 26 MRS detected, 19.2% were coagulase-positive, 73.1% produced haemolysins, 26.9% produced DNase, 96.2% were gelatinase-positive, 69.2% were lipase-positive and 38.5% of the isolates were able to express biofilm *in vitro*. *Ica*A and *ica*D genes were both present in 38.5% of the isolates, but none was *bap*-positive. Only 11.5% of the isolates were typeable for *agr*. Antimicrobial co-resistance ranged from 0% (Chloramphenicol, Vancomycin) to 92.3% (Nalidixic acid). Bacteriophages were present in 34.6% of the isolates and none produced bacteriocins.

The high frequencies of virulence traits present combined with the high antimicrobial co-resistance profiles observed, suggest that these isolates may represent a serious problem with major public health implications, re-enforcing the importance of the wide concept of "One Health".

Keywords: virulence traits, staphylococci, animal, biofilm, methicillin resistance